Protocol for Heat-Inactivation of Plasma and Serum Samples (Montefiori Lab) July 2008

I. INTRODUCTION

Serum and plasma samples must be heat-inactivated to destroy complement prior to performing neutralizing antibody assays. Complement is comprised of a set of approximately 20 blood proteins that mediate a variety of functions in the body [1]. One of these functions is to kill microbes by complement-mediated lysis. Human complement does not lyse human cells or HIV-1 (rabbit and rodent complement will) because both contain regulatory proteins on their surface (e.g., CD35, CD46, CD55, CD59) that inhibit the terminal complement pathway and formation of the membrane attack complex [2]. The virus obtains these regulatory proteins as it buds from the plasma membrane of infected cells [3, 4]. Nonetheless, human complement will deposit on the virus surface in the form of activated fragments of complement component C3 (e.g., C3b and C3d), which are part of the early activation pathway that is not affected by the regulatory proteins mentioned above. HIV-1 can activate the complement system through specific sites on gp120 and gp41 and the activation is further facilitated by antibodies to both envelope glycoproteins [3].

Complement activation by antibody is mediated by the Fc region of the antibody molecule. Deposition of activated complement fragments on the virus surface can target the virus to complement receptors on several $CD4^+$ T cell lines used for neutralization assays (MT-2, CEMx174, M7-Luc). The most common complement receptor on these cells that the virus uses is CR2 (CD21) [5-7]. An important consequence of complement deposition on the virus is infection-enhancement. Infection-enhancement is most evident in the presence of gp120-specific and gp41-specific antibodies and may mask the activity of neutralizing antibodies [4]. It is not clear that infection-enhancing antibodies detected in this assay are relevant to natural target cells *in vivo* [4, 8, 9]. Enhancement may be measured in independent assays if desired [8, 9].

Complement activation is not known to be an issue for PBMC assays because normal $CD4^+$ T cells do not express a sufficient level of complement receptors. Both serum and plasma contain complement. The complement system can be destroyed by heat-inactivation at 56°C.

II. DEFINITIONS

ACD: Acid Citrate Dextrose

EDTA: Ethylenediaminetetraacetic acid

ID: Identification

III. REAGENTS AND MATERIALS

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality may be used when necessary.

Cryogenic vials, 1.5 ml sterile screw cap, frosted label
Sarstedt Brand Products

Sterile gauze pads
VWR Scientific

Disposable pipettes, sterile, individually wrapped
Falcon/VWR
1 ml pipettes
5 ml pipettes

Instrumentation:

Biological Safety Cabinet
Baker Co.

Water bath
Precision Scientific

Centrifuge and Microcentrifuge, Maximum rotational speed = 14,000 rpm
Eppendorf
18 place standard rotor F-45-18-11 for 1.5 ml microcentrifuge tubes

PipetteAid XP
Drummond Scientific Co.

Specimens:

Specimens can be either serum or plasma and are stored in 1.5 ml sterile polypropylene cryovials. Serum is obtained from coagulated blood, which is collected in a red-top vacutainer tube. Plasma is obtained from anti-coagulated blood, where clotting is prevented by adding either heparin, EDTA, or ACD to the tubes prior to blood collection. Collection tubes for anti-coagulated blood can be obtained commercially and are color-coded green (heparin), yellow (ACD), and purple (EDTA).

Serum is preferred for neutralizing antibody assays. Anticoagulants in plasma are problematic, especially when heparin is used (some forms of heparin have potent and strain-specific antiviral activity), since they are all toxic to cells as plasma dilutions lower than 1:60.

IV. PROTOCOL

Heat Inactivation Procedure

1. Thaw samples at room temperature. This may be accomplished in a short period of time by placing the tubes in a water bath at ambient temperature.

2. Gently mix the samples after thawing and centrifuge briefly (pulse for approximately 5 seconds) in a microcentrifuge to assure that no sample remains adhered to the inside cap or sides of the tube.

> *NOTE 1: Proteins will separate from serum and settle on the bottom of the tube after a freeze-thaw cycle. It is important to mix the samples to redistribute the proteins evenly throughout the sample prior to heat-inactivation.*

3. Place the tubes in a 56°C water bath, allowing the water level to reach the top of the sample volume but not touching the rim of the cap of the tube. Incubate for 1 hour for samples which were not previously heat-inactivated. Incubate for 30-45 minutes for samples that were previously heat-inactivated for 30 minutes or less.

> *NOTE 2: The water bath should be turned on at least 1 hour before use to assure the temperature has stabilized.*
>
> *NOTE 3: Caution! Temperatures above 58°C will turn serum and plasma into the solid phase – a process that cannot be reversed and essentially destroys the sample permanently.*

4. Remove the tubes from the water bath and wipe the outside of the tubes with a clean gauze pad.

5. Gently mix the samples to collect condensation on the inside surface and centrifuge the samples briefly (pulse for approximately 5 seconds) to assure that no sample remains adhered to the inside cap or sides of the tube.

6. Mark each tube with a triangle on the cryogenic tube label to indicate the heat inactivation process has been completed.

7. Store the samples at 4°C when assays are to be performed within 2 weeks. Store the tubes at -80°C when assays are delayed for >2 weeks.

8. Log the date, time, temperature, and number of samples processed in a heat-inactivation log.

V. REFERENCES

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